IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Atty. Docket: KATSUKI=1

In re Application of:	)	Art Unit: 1615
Hisakazu KATSUKI	)	
Appln. No.: 09/914,066	)	Art Unit: 1615
Nationalized: August 23, 2001	)	Examiner: Charesse L. Evans
I.A. No.: PCT/JP00/00862	)	Confirmation No.: 8579
I.A. Date: February 16, 2000	)	Washington D.C.
For: SEAM SOFT CAPSULE PREPARATION CONTAINING DIHYDROBENZOFURAN DERIVATIVE	)	

DECLARATION UNDER 37 CFR 1.132

Honorable Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Hisakazu KATSUKI, a Japanese citizen, hereby declare that I am an inventor of the above-identified patent application, and that I graduated for the Faculty of Pharmaceutical Sciences at Kumamoto University, Kumamoto-ken, Japan, and received a Master of Pharmacy in March, 1995, at Kumamoto University, and received a Doctoral degree in Pharmacy in September, 2001, at the University of Shizuoka, Graduate School of Pharmaceutical Sciences.

I also declare that I have been employed by Chugai Pharmaceutical Co., Ltd. since April, 1995, and currently I work as a researcher in formulation technology research for the Formulation Technology Research Department of the

company.

I declare further that I have read the Office Action issued against the subject patent application mailed on April 16, 2004 and have noted the Examiner's allegation that Claims 6-14, 16 and 19-20 of the patent application are rejected under 35 U.S.C.103(a) as being unpatentable over Lacy et al (US 6,096,338) in view of Amey et al (US 6,080,426).

I conducted the following experiment using 4,6-di-tert-butyl-2,2-di-n-pentyl-5-hydroxy-2,3-dihydrobenzofuran, BO-653, which is claimed in Claims 19 and 20 of the subject patent application, and 4,4'-[(1-methylethylidene)bis(thio) bis-[2,6-bis(1,1-dimethylethyl)phenol], Probucol, which is not claimed in the subject patent application and is referred to in the Office Action as being indicated at column 13, line 4 of Lacy et al, in order to demonstrate that dissolving BO-653 in soybean oil is greatly advantageous over dissolving Probucol in soybean oil in terms of drug stabilization.

I declare that the results are true and correct to the best of my knowledge.

Method:

400 mg each of original drugs, BO-653 and Probucol, were placed into individual 15.5 cc glass bottles. BO-653 was in a viscous liquid state, while Probucol being in a powdery solid state, at a room temperature.

Separately, 5 wt% solutions were prepared by dissolving each of original drugs, BO-653 and Probucol, in soybean oil to a concentration of 5 wt%. The reason why the

concentration, 5 wt%, was employed is that the solubility of probucol in soybean oil is about 5 %. 400 mg each of the solutions were placed into individual 15.5 cc glass bottles.

All the non-sealed bottles respectively containing the four samples were placed in an air-filled thermostatic chamber controlled at 80℃ and allowed to stand for 17 hours. After removing the bottles from the chamber, the contents in the bottles were each analyzed by HPLC to determine the percentage of the amounts of BO-653 or Probucol remaining without being decomposed, to those not having been subjected to this accelerated decomposing test.

The HPLC conditions were as follows:

Column: YMC-Pack ODS AM303 (250x4.6mm, 5μm)

Mobile phase: Acetonitrile/2-propanol/water (800:190:10)

Flow rate: 1 mL/min

Column temperature: Room temperature

Detection wavelength: 300 nm for BO-653 and 241 nm for Probucol

Results:

The results were summarized in Table A, below.

Table A

BO-653 and Probucol(%) Remained After Accelerated Decomposing Test

Sample	Original Drug	Solution in Soybean Oil
BO-653	64.6	87.9
Probucol	99.4	99.0

As can be seen from the table, only 64.6% of BO-653 remains not decomposed under the accelerated decomposing conditions when in the form of original drug, while 87.9% of the drug remains not decomposed under the same conditions when dissolved in soybean oil, indicating that BO-653 is effectively stabilized by dissolving in soybean oil. In contrast, Probucol is stable both in the form of powdery solid and in soybean oil solution under the same conditions, indicating that the combination of Probucol with soybean oil does not provide any advantage in terms of stabilization.

I hereby further declare that all statements made herein are to my own knowledge and belief true, and that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

September 13, 2004 Hisakazu Katsuki

Hisakazu KATSUKI

neal macrophage preparations. Peritoneal macrophages were prepared according to the method of Adams (21) by using Brewer's thioglycolate medium (Difco). Rabbits and mice were housed in animal rooms for at least 1 week before the experiments, with free access to food and water. The animals were treated in accordance with Chugai Pharmaceutical's ethical guidelines for animal care, handling, and termination. These guidelines meet the generally accepted international criteria of humane treatment, sparing the animals needless pain and suffering and ensuring that the experiments conducted are of actual scientific benefit to humankind.

Chemiluminescence Assay. Linoleic acid peroxyl radicals were generated by autoxidation and detected by chemiluminescence using a *Cypridina* luciferin analog [2-methyl-6-(*p*-methoxyphenyl)-3,7-dihydroimidazo[1,2-*a*]pyrazin-3-one (MCLA)] (22). A solution of 1 mM linoleic acid in 1-butanol containing 10 μM MCLA was incubated for 10 min at 37°C under air. The chemiluminescence was induced by the fast reaction of MCLA with singlet oxygen generated by linoleic acid peroxyl radicals. The quenching of the chemiluminescence was evaluated as the change in chemiluminescence intensity after addition of the compound.

Oxidation of LDL. LDL (density 1.019–1.063 g/ml) was isolated by sequential ultracentrifugation from rabbit plasma (23). LDL (200 μg/ml) and the test compound were incubated at 37°C for 24 hr with 10 μM $CuSO_4$ or 40 μg/ml soybean lipoxygenase. Lipid peroxides were determined as thiobarbituric acid-reactive substances (TBARS) (22). For the degradation assays, LDL was labeled with $Na^{125}I$ by using the iodine monochloride procedure (24). ^{125}I-LDL was incubated at 37°C for 24 hr with 10 μM $CuSO_4$, and the degradation was carried out by incubating mouse peritoneal macrophages in RPMI 1640 medium containing 10 μg/ml LDL at 37°C for 5 hr, following which the trichloroacetic acid-soluble non-iodide radioactivity was determined.

Diets and Administration of Compounds. The high-fat diet (1.25% cholesterol) was prepared according to Nishina *et al* (25). The diets used in this paper were made by Clea Japan, and the indicated concentration of compound in the diet was confirmed by HPLC as described below. C57BL/6J mice were maintained for 28 weeks on a high-fat diet containing 0.5% of the test compound or cellulose; LDL-receptor knockout mice were maintained for 13 weeks or 21 weeks on the diet. WHHL rabbits were maintained for 6 months on a normal diet (CR3) without added antioxidants or with 1% probucol or 0.2% or 0.5% BO-653.

Measurement of Lipoprotein Profile and Concentration of the Compound. Plasma lipoprotein profiles were evaluated as cholesterol concentrations of fractions by gel-permeation chromatography (GPC). Ten microliters of blood was obtained from a dorsal metatarsal vein and was quickly diluted into saline. Then the diluted plasma sample was obtained by centrifugation and analyzed by GPC for lipoprotein profile and by auto-analyzer (COBAS FARAII, Hoffmann–La Roche) for lipid composition. The concentrations in diets, plasma, and tissues were determined by reverse-phase HPLC of the methanol-soluble fraction of diet, plasma, and tissue homogenate samples.

Evaluation of Atherosclerotic Lesions and Xanthoma Score. The atherosclerotic lesion area of WHHL rabbits was evaluated as the area of fatty region on aortic inner surfaces. Frozen cross sections of aortic valves or aortic arches were stained with Sudan IV and counterstained with hematoxylin. The atherosclerotic lesions were evaluated as the mean area of Sudan IV-positive regions in the aortic valve cross sections (26) or aortic cross sections (four sections of 6-mm thickness at 1-mm intervals). All areas were determined by SigmaScan Pro software (Jandel

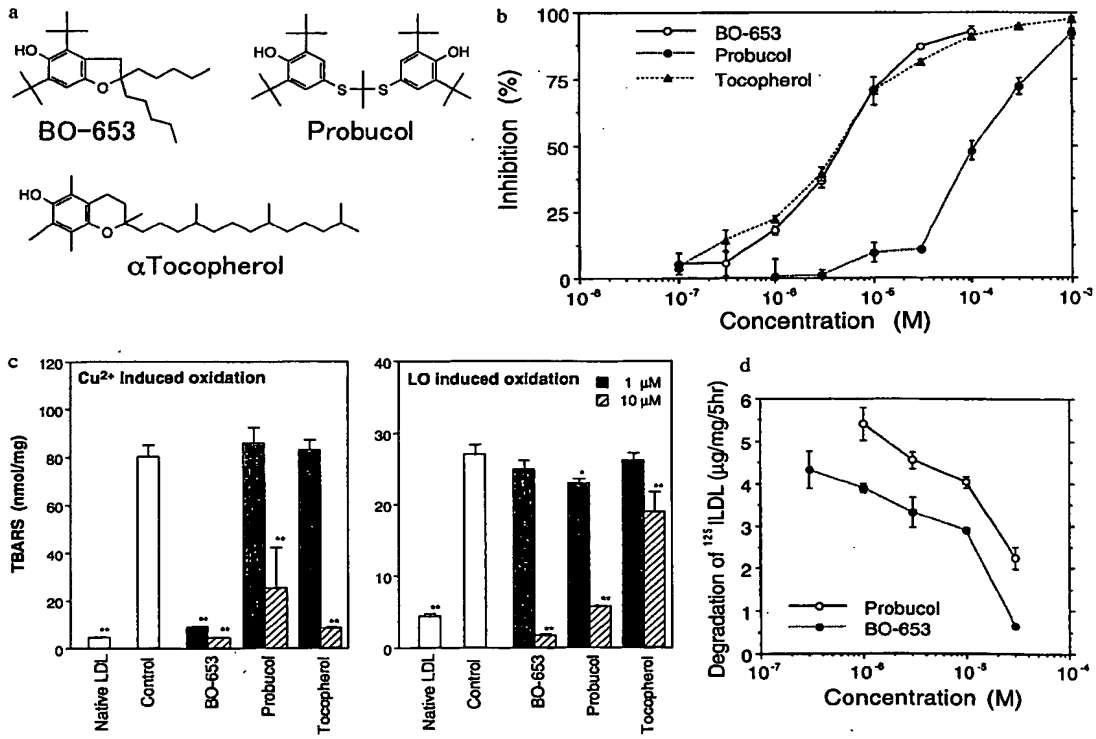
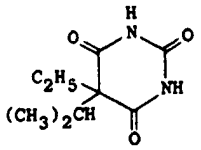


FIG. 1. Properties of the antioxidant BO-653. (*a*) Structure of BO-653, 2,3-dihydro-5-hydroxy-2,2-dipentyl-4,6-di-*tert*-butylbenzofuran, and the structures of the antioxidants probucol and α-tocopherol. (*b*) Inhibition of BO-653 on chemiluminescence induced by linoleic acid peroxyl radicals. Linoleic acid peroxyl radicals were generated by autoxidation and detected by chemiluminescence using MCLA. (*c*) Inhibition of BO-653 on rabbit LDL oxidation. LDL and the test compound were incubated at 37°C for 24 hr with $CuSO_4$ or soybean lipoxygenase (LO) and the lipid peroxides were determined as TBARS. (*d*) Inhibition by BO-653 of ^{125}I-LDL degradation induced by oxidation. ^{125}I-LDL was incubated at 37°C for 24 hr with $CuSO_4$ and the degradation was carried out by incubation with mouse peritoneal macrophages. All results are shown as mean ± SD of triplicate (*b* and *c*) or duplicate (*d*) samples. *, $P < 0.05$; **, $P < 0.01$.

(1965). Consists of pristinamycins I_A, I_B, I_C, which are identical to vernamycins B_α, B_β, B_γ (*q.v.*) respectively, and pristinamycins II_A, II_B, which are identical to virginiamycin M_1, (*q.v.*), and 26,27-dihydrovirginiamycin M_1. Separation of components and structure: Preud'homme *et al., Bull. Soc. Chim. France* **1968,** 585. Nomenclature: Crooy, De Neys, *J. Antibiot.* **25,** 371 (1972).

THERAP CAT: Antibacterial.

7759. Probarbital. *5-Ethyl-5-(1-methylethyl)-2,4,6-(1H,3H,5H)-pyrimidinetrione; 5-ethyl-5-isopropylbarbituric acid;* Ipral. $C_9H_{14}N_2O_3$; mol wt 198.22. C 54.53%, H 7.12%, N 14.13%, O 24.22%. Prepn: Thorp, U.S. pats. **1,255,951; 1,576,014** (1918, 1926). Toxicity data: R. H. Fitch, A. L. Tatum, *J. Pharmacol. Exp. Ther.* **44,** 325 (1932).



Needles, mp 197-198°. Very slightly sol in cold water, more readily sol in hot water; easily sol in alcohol, ether.

Calcium salt trihydrate, $C_{18}H_{26}CaN_4O_6 \cdot 3H_2O$, crystals. Slightly bitter taste. One gram dissolves in about 40 ml water. Practically insol in alcohol. Aq solns are alkaline to litmus. Solns are unstable and pptn occurs on boiling. LD_{50} in rats, rabbits (mg/kg): 110, 110 i.p. (Fitch, Tatum).

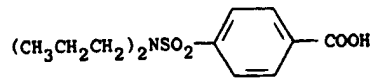
Sodium salt, $C_9H_{13}N_2NaO_3$, hygroscopic powder. Freely sol in water; slightly sol in alc. Practically insol in ether, chloroform. Aq solns are alkaline to litmus. Solns are unstable and pptn occurs on boiling.

Caution: May be habit forming. This is a controlled substance (depressant) listed in the U.S. Code of Federal Regulations, Title 21 Part 1308.13 (1987).

THERAP CAT: Sedative, hypnotic.

THERAP CAT (VET): Sedative, hypnotic.

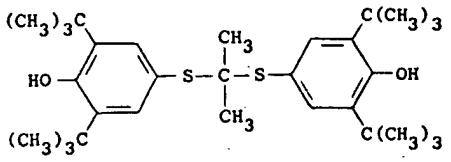
7760. Probenecid. *4-[(Dipropylamino)sulfonyl]benzoic acid; p-(dipropylsulfamoyl)benzoic acid; p-*(dipropylsulfamyl)benzoic acid; Benemid; Probecid; Proben. $C_{13}H_{19}NO_4S$; mol wt 285.36. C 54.72%, H 6.71%, N 4.91%, O 22.43%, S 11.23%. Prepn from *p*-carboxybenzenesulfonyl chloride and dipropylamine: C. S. Miller, U.S. pat. **2,608,507** (1952 to Sharp & Dohme). Study of metabolites: Z. H. Israili *et al., J. Med. Chem.* **15,** 709 (1972). Pharmacokinetics in man: R. F. Cunningham *et al., Clin. Pharmacokinet.* **6,** 135 (1981). Comprehensive description: A. A. Al-Badr, H. A. El-Obeid in *Analytical Profiles of Drug Substances* **vol. 10,** K. Florey, Ed. (Academic Press, New York, 1981) pp 639-663.



Crystals from dilute alcohol, mp 194-196°. uv max (0.1*N* NaOH): 242.5 nm. pKa 5.8. Slightly bitter taste, pleasant aftertaste. Sol in chloroform, in dil solns of NaOH buffered to pH 7.4. Nearly insol in water. LD_{50} orally in rats: 1.6 g/kg.

THERAP CAT: Uricosuric.

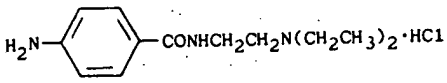
7761. Probucol. *4,4'-[(1-Methylethylidene)bis(thio)]bis-[2,6-bis(1,1-dimethylethyl)phenol]; 4,4'-(isopropylidenedithio)bis[2,6-di-tert-butylphenol];* acetone bis(3,5-di-*tert*-butyl-4-hydroxyphenyl)mercaptole; DH-581; Biphenabid; Bisbid; Bisphenabid; Lorelco; Lurselle; Sinlestal. $C_{31}H_{48}O_2S_2$; mol wt 516.84. C 72.04%, H 9.36%, O 6.19%, S 12.41%. Prepn: M. B. Neuworth, Fr. pat. **1,561,853;** *eidem,* U.S. pat. **3,576,883** (1969, 1971 both to Consolidation Coal Co.); and use as a cholesterol-lowering agent: J. W. Barnhart, P. J. Shea, U.S. pat. **3,862,332** (1975 to Dow). Prepn and activity studies: M. B. Neuworth *et al., J. Med. Chem.* **13,** 722 (1970). Pharmacological studies: J. W. Barnhart *et al., Am. J. Clin. Nutr.* **23,** 1229 (1970); Drake *et al., Circulation* **40,** Suppl. 3, 73 (1969). Clinical studies: *eidem, Metab. Clin. Exp.* **18,** 916 (1969); Kalams *et al., Curr. Ther. Res.* **13,** mechanism of action, clinical efficacy and safety: *Am. J. Cardiol.* **57,** 1H-54H (1986).



White crystalline solid from ethanol, mp 124.5-126°; fine, yellow crystals from isopropanol, mp 125-126.5°.

THERAP CAT: Antihyperlipoproteinemic.

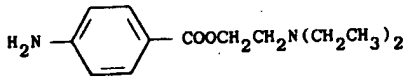
7762. Procainamide Hydrochloride. *4-Amino-N-[2-(diethylamino)ethyl]benzamide monohydrochloride;* procaine amide hydrochloride; Amidoprocain; Amisalin; Novocamid; Novocainamid; Procamide; Procan-SR; Procapan; Procardyl; Promide; Pronestyl hydrochloride; Supicaine Amide hydrochloride. $C_{13}H_{22}ClN_3O$; mol wt 271.79. C 57.45%, H 8.16%, Cl 13.05%, N 15.46%, O 5.89%. Prepn: M. Yamazaki *et al., J. Pharm. Soc. Japan* **73,** 294 (1953); Y. Tashika, M. Kuranari, *ibid.* 1069. Comprehensive description: R. B. Poet, H. Kadin in *Analytical Profiles of Drug Substances* **vol. 4,** K. Florey, Ed. (Academic Press, New York, 1975) pp 333-383.



Crystals, mp 165-169°. uv max: 278 nm. Freely sol in water. Sol in alc; slightly sol in chloroform; very sparingly sol in benzene, ether. The pH of a 10% aq soln is 5.5. Commercially available aq solns are preserved with 0.9% benzyl alcohol and 0.09% sodium bisulfite.

THERAP CAT: Cardiac depressant (antiarrhythmic).

7763. Procaine. *4-Aminobenzoic acid 2-(diethylamino)-ethyl ester; p-*aminobenzoyldiethylaminoethanol; 2-diethylaminoethyl *p*-aminobenzoate. $C_{13}H_{20}N_2O_2$; mol wt 236.30. C 66.07%, H 8.53%, N 11.86%, O 13.54%. Benzoic acid derivative with anesthetic activity. Prepn: A. Einhorn, U.S. pat. **812,554** (1906); *idem, Ann.* **371,** 125 (1909); A. Einhorn, E. Uhlfelder, *ibid.* 131. CNS effects: C. G. Peterson, *Anesthesiology* **16,** 976 (1955). Intravenous pharmacokinetics in humans: A. B. Seifen *et al., Anesth. Analg. (Cleveland)* **58,** 382 (1979). Clinical evaluation as anti-arrhythmic and cough suppressant during anesthesia: D. S. Thompson *et al., Am. J. Surg.* **138,** 798 (1979). Stabilization of vascular smooth muscle *in vitro:* K. Kitamura *et al., Drugs Exp. Clin. Res.* **12,** 773 (1986). Toxicity data: W. C. North, K. F. Urbach, *J. Am. Pharm. Assoc. Sci. Ed.* **45,** 382 (1956); E. I. Goldenthal, *Toxicol. Appl. Pharmacol.* **18,** 185 (1971).



Hygroscopic, anhydr plates, tablets from ligroin or ether, mp 61°. When freshly precipitated, one gram dissolves in 200 ml water. Sol in alc, ether, benzene, chloroform. LD_{50} in mice (mg/kg): 195 i.p.; 45 i.v. (North, Urbach).

Dihydrate, needles from aq alc, mp 51°. Slightly bitter taste; applied to the tongue causes transitory numbing sensation.

Nitrate, $C_{13}H_{21}N_3O_5$, crystals, mp 100-102°. Sol in water, alcohol. The aq soln is neutral. Particularly useful with silver nitrate because no precipitate forms.

Butyrate, $C_{17}H_{28}N_2O_4$, *Probutylin.* Hygroscopic crystals. Soluble in water, alcohol, vegetable oils.

Borate, $C_{13}H_{25}B_5N_2O_{12}$, *Borocaine.* Small, monoclinic, tabular crystals, mp 165-166°. One gram dissolves in about 4 ml water. Sol in alcohol. Insol in benzene, chloroform, ether. Aq solns are alkaline, may be sterilized by brief boiling.